

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 39/385, 39/00, 39/39	A1	(11) International Publication Number: WO 96/40242 (43) International Publication Date: 19 December 1996 (19.12.96)
(21) International Application Number: PCT/EP96/02436 (22) International Filing Date: 4 June 1996 (04.06.96) (30) Priority Data: 08/472,639 7 June 1995 (07.06.95) US 9512827.8 23 June 1995 (23.06.95) GB 9513443.3 1 July 1995 (01.07.95) GB 9525657.4 15 December 1995 (15.12.95) GB (71) Applicant (for all designated States except US): SMITHKLINE BEECHAM BIOLOGICALS S.A. [BE/BE]; Rue de l'Institut 89, B-1330 Rixensart (BE). (72) Inventors; and (75) Inventors/Applicants (for US only): SLAOUI, Moncef, Mohamed [BE/BE]; SmithKline Beecham Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE). HAUSER, Pierre [BE/BE]; SmithKline Beecham Biologicals S.A., Rue de l'Institut 89, B-1330 Rixensart (BE). (74) Agent: DALTON, Marcus, Jonathan, William; SmithKline Beecham, Corporate Intellectual Property, SB House, Great West Road, Brentford, Middlesex TW8 9BD (GB).		(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: VACCINE COMPRISING A POLYSACCHARIDE ANTIGEN-CARRIER PROTEIN CONJUGATE AND FREE CARRIER PROTEIN		
(57) Abstract The present invention relates to combination vaccines comprising a conjugated polysaccharide antigen linked to a carrier protein, and wherein the carrier protein is also present as a free antigen in the vaccine composition, characterised in that the ratio of polysaccharide to protein is from 1:0.3 to 1:2. In particular, the vaccine composition of the present invention relates to a multivalent vaccine, that is a vaccine for the amelioration or treatment of more than one disease state. The present invention also relates to the production and use of such a vaccine in medicine.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

VACCINE COMPRISING A POLYSACCHARIDE ANTIGEN - CARRIER PROTEIN CONJUGATE AND FREE CARRIER PROTEIN

The present invention relates to combination vaccines comprising a conjugated polysaccharide antigen linked to a carrier protein, and wherein the carrier protein is also present as a free antigen in the vaccine composition. In particular the vaccine composition of the present invention relates to a multivalent vaccine, that is a vaccine for the amelioration or treatment of more than one disease states. The present invention also relates to the production and use of such a vaccine in medicine.

Vaccines that utilise polysaccharides are known in the art. For example a vaccine for the prevention of *Haemophilus influenzae* b (Hib) infections are based on the capsular polysaccharide (PRP) conjugated with a carrier protein. Typically the carrier protein is a diphtheria or tetanus toxoid. It has also been suggested to produce a vaccine for the prevention of *Streptococcus pneumonia* which is based on capsular polysaccharide conjugated to a carrier protein such as tetanus toxoid or diphtheria toxoid. Examples of such conjugate vaccine antigens are disclosed in US 4 365 170 US 4 673 574 EP 208 375 EP 477508 and EP 161 188.

It is desirable to administer such conjugate vaccines with other antigens or vaccines at the same time and this can involve multiple injections. Problems associated with multiple injections include a more complicated administration procedure and a large total injection volume. This is a particularly acute problem when the vaccine is intended for infants.

It has therefore been proposed to produce combination vaccines. One well known combination vaccine provides protection against Diphtheria, tetanus and B. pertussis infections. This vaccine comprises a pertussis component (either killed whole cell B. pertussis or acellular pertussis which typically consists of up to three antigens - PT, FHA and 69kDa) and toxoided diphtheria and tetanus toxin. Such vaccines are often referred to as DTPw (whole cell) or DTPa (acellular).

It would be desirable to add polysaccharide conjugate vaccines to such a combination. However we have found that simple mixing of the components results in a reduction of antibody titres to the polysaccharide component and that the persistence of protective levels of such antibody titres is reduced. Thus the immunogenicity of conjugate PRP Hib polysaccharide, to detoxified Tetanus Toxoid (TT) has been observed to decrease in infants immunised with TT-PRP conjugate combined to DTPa as compared to infants immunised with TT-PRP conjugate and DTPa concomitantly, but at separate sites.

In the monovalent situation, ie when a vaccine comprises just a single polysaccharide (PS) conjugated to a carrier protein, the higher the protein content of

the conjugate the higher the antibody response to the polysaccharide. This is thought to be because induction of a T cell dependent polysaccharide specific antibody response requires the covalent coupling of a protein carrier to the polysaccharide. The protein carrier needs to be present at concentrations (ie PS to protein ratio) such that a good TH cell response to the carrier is induced, and that this TH response can recognise PS specific B cell clones expressing peptides derived from the covalently coupled carrier protein.

Typically the prior art monovalent vaccines have a ratio of polysaccharide: protein carrier of 1:3 (weight:weight).

Surprisingly and in complete contrast to the position in the monovalent vaccine, the present inventors have discovered that when the polysaccharide conjugate component is part of a multivalent vaccine comprising a free form of the carrier protein, the immunogenicity of the polysaccharide component is improved by reducing the protein content of the conjugate antigen.

Accordingly the present invention provides a combined vaccine containing a polysaccharide antigen conjugated to a carrier protein and wherein the carrier protein is also present as free antigen characterised in that the ratio of polysaccharide to carrier protein is from approximately 1:0.3 to 1:2. A preferred ratio is 1:0.5 to 1:1.5, typically in the order of 1:1.

The polysaccharide conjugate may be prepared by any known coupling technique. For example the polysaccharide can be coupled via a thioether linkage. This conjugation method relies on activation of the polysaccharide with 1-cyano-4-dimethylamino pyridinium tetrafluoroborate (CDAP) to form a cyanate ester. The activated polysaccharide may thus be coupled directly or via a spacer group to an amino group on the carrier protein. Preferably, the cyanate ester is coupled with hexane diamine and the amino-derivatised polysaccharide is conjugated to the carrier protein using heteroligation chemistry involving the formation of the thioether linkage. Such conjugates are described in PCT published application WO93/15760 Uniformed Services University.

The conjugates can also be prepared by direct reductive amination methods as described in US 4365170 (Jennings) and US 4673574 (Anderson). Other methods are described in EP-0-161-188, EP-208375 and EP-0-477508.

A further method involves the coupling of a cyanogen bromide activated polysaccharide derivatised with adipic acid hydrazide (ADH) to the protein carrier by Carbodiimide condensation (Chu C. et al Infect. Immunity, 1983 245 256).

Preferably the carrier protein is an antigen derived from a Tetanus, or Diphtheria preferably tetanus toxoid or Diphtheria toxoid. The carrier may also be

derived from Bordetella. Typically the antigen is non toxic derivative of a toxin from Tetanus or Diphtheria.

Preferably the compositions of the invention contain a suitable adjuvant.

A preferred adjuvant is MPL (3-deacylated monophosphoryl lipid A, also
5 known as 3D-MPL). 3D-MPL is known from GB 2 220 211 (Ribi) as a mixture of
3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains and is
manufactured by Ribi Immunochem, Montana. A preferred form of MPL is
disclosed in International Patent Application 92/116556.

Another adjuvant which may be used in the present invention is known as
10 QS21. QS21 is a Hplc purified non toxic fraction of a saponin from the bark of the
South American tree Quillaja saponaria molina and its method of its production is
disclosed (as QA21) in US patent No. 5,057,540.

Combinations of QS21 and 3 D-MPL are known to produce a synergistically
effective vaccine formulation and are described in International Patent application
15 WO 94/00153.

Often the Hib component vaccines of the invention will not require any
specific carrier and be formulated in an aqueous or other pharmaceutically
acceptable buffer. In some cases it may be that the entire vaccine of the present
invention will be presented in an oil in water emulsion, or other suitable vehicle,
20 such as for example, liposomes, microspheres or encapsulated antigen particles.

The vaccine formulations may contain further antigens. Antigen or
antigenic compositions known in the art can be used in the compositions of the
invention, including antigen or antigenic compositions derived from HIV-1, (such
as gp120 or gp160), human or animal herpes viruses, such as gD or derivatives
25 thereof or Immediate Early protein such as ICP27 from HSV1 or HSV2,
cytomegalovirus (especially human)(such as gB or derivatives thereof), Varicella
Zoster Virus (such as gpI, II or III), or from a hepatitis virus such as hepatitis B
virus for example Hepatitis B Surface antigen or a derivative thereof, hepatitis A
virus, hepatitis C virus and hepatitis E virus, or from other viral pathogens, such as
30 Respiratory Syncytial virus, human papilloma virus or Influenza virus, or polio
virus such as Inactivated Polio Virus (IPV) or derived from bacterial pathogens such
as Diphtheria (D), Tetanus (T), Salmonella, Neisseria, particularly Neisseria
Meningitis A, B, or C. Borrelia (for example OspA or OspB or derivatives
thereof), or Chlamydia, or Bordetella for example P.69, PT and FHA from
35 B.pertussis (P), or derived from parasites such as plasmodium or Toxoplasma.

Particularly preferred vaccines of the invention include, DTPa Hib; and
DTPa Hib Hep B; and DTPa Hib Hep B IPV.

It will be appreciated that in the combination vaccine of the invention the Hib component may be formulated with the other antigens just prior to administration. Thus a lyophilised Hib component may be reconstituted prior to use, by mixing with the aqueous formulation of other antigens.

5 The above combinations may optionally include a component which is protective against Hepatitis A.

Suitable components for use in such vaccines are already commercially available and details may be obtained from the World Health Organisation. For example the IPV component may be the Salk inactivated polio vaccine. The
10 Diphtheria, Tetanus and Pertussis vaccine may comprise an acellular product such as Infanrix DTPa (SmithKline Beecham Biologicals). The component affording protection against Hepatitis A is preferably the product known as 'Havrix' (SmithKline Beecham Biologicals) which is a killed attenuated vaccine derived from the HM-175 strain of HAV [see 'Inactivated Candidate Vaccines for Hepatitis A' by
15 F.E. Andre, A Hepburn and E.D'Hondt, *Prog Med. Virol.* Vol 37, pages 72-95 (1990) and the product monograph 'Havrix' published by SmithKline Beecham Biologicals (1991)]. The Hepatitis B component may comprise the 'S' antigen as in 'Engerix-B'.

Advantageously the Haemophilus Influenzae B vaccine or combination
20 vaccine according to the invention is a paediatric vaccine.

Vaccine preparation is generally described in Vaccine Design - the subunit and Adjuvant approach edited by Michael F Powell and Mark Newman, Plenum Press. Encapsulation within liposomes is described, for example, by Fullerton, US Patent 4,235,877. Conjugation of proteins to macromolecules is disclosed, for
25 example, by Likhite, US Patent 4,372,945 and by Armor et al., US Patent 4,474,757.

The amount of antigen in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in
30 typical vaccinees. Such amount will vary depending on which specific immunogens are employed. Generally it is expected that each dose will comprise 1-1000ug of total immunogen, preferably 2-100ug, most preferably 4-40ug. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of antibody titres and other responses in subjects. Following an initial vaccination, subjects may receive one or two booster injections at about 4 weeks intervals.

35 The following examples will illustrate the invention.

Examples

Example 1a) Vaccine comprising DTPa - Hib PRP-TT - and HBsAg.

5 Production of Hib TT conjugate

Materials and Methods

Materials

10 The Hib PRP is extracted and purified from inactivated cell cultures. The purified material meets the WHO and US specifications in terms of residual protein, nucleic acid, endotoxin, structural sugars and molecular size distribution.

Tetanus toxoid produced by Behringwerke meets also the WHO specifications. The material is further purified by acid pH precipitation and gel
15 filtration chromatography to isolate the monomeric form (150 kDa) of TT.

Activation and Coupling Chemistry

20 mg of Hib PRP were dissolved in 4 ml of 2 M NaCl.

150 mcl of CDAP (1-cyano-4-dimethylamino-pyridinium tetrafluoroborate)
20 was added to the polysaccharide solution (from a 100 mg/ml stock solution in acetonitrile). One minute later, 300 mcl of triethylamine 0.2 M was added. The activation of the polysaccharide was performed during 2 minutes at 0°C at pH 9.5-10.

20 mg or 40 mg of the Tetanus toxoid (initial PRP/Protein ratio of 1/1 or
25 1/2) at a concentration of 15 mg/ml was added and the coupling reaction was performed at 25°C for 1 hour. Then the reaction was quenched for 1 hour at 25°C with 3 ml of 1 M glycine solution pH 5.0 and then overnight at 4°C.

The conjugate is purified by gel filtration chromatography using a Sephacryl HR 500 gel filtration column equilibrated with 0.2 M NaCl. The carbohydrate and
30 protein content of the eluted fractions was determined. The conjugate was pooled in and sterile filtered. The PRP/protein ratio and free PS content in the conjugate preparation were determined.

Example 1b)**DTP Hib PRP TT HBsAg Combination Vaccine Formulation**

5 Formulation of DTPa (comprising Diphtheria toxoid, Tetanus toxoid, pertussis toxoid, pertactin and FHA) with or without Hepatitis B vaccines are known in the art. For example such a vaccine is disclosed in International Patent application No WO 93/24148. The Conjugate as described in example 1 was added to the vaccine and mixed prior to injection for immunogenicity studies.

10

In the present studies the following steps were undertaken:

- adjustment of phenoxy-ethanol (bacteriostatic) content to 5 mg/ml
- adjustment of pH at 6.1 ± 0.1
- 15 • adjustment of PRP concentration up to 100 mcg/ml
- addition of DTPaHBV (0.5 ml) to 0.1 ml of the PRP-CDAP-TT solution.

The final composition of the vaccine was for one dose:

20	• PRP'P	10 mcg
	• D	25 Lf
	• T	10 Lf
	• PT	25 mcg
	• FHA	25 mcg
25	• 69K	8 mcg
	• Hep B	10 mcg
	• A1 (OH) ₃ Behring	0.35 mg
	• A1 (OH) ₃ Superpos	0.15 mg
	• A1PO ₄ Superpos	0.20 mg
30	• NaCl	150 mM
	• 2φEt-OH	3 mg (→5 mg/ml)
	• pH	6.1 ± 0.1
	• Vol	0.6 ml

Example 2

Immunogenicity

5 The immunogenicity of Haemophilus Influenzae type B derived polysaccharide:protein (weight:weight) ratios (1:3, 1:2 and 1:1), using two different coupling chemistries, is compared after a first and second inoculation in young OFA rats.

10 The data obtained at 28 days after the first vaccine inoculation clearly show an improved anti PS antibody titre correlating with a decreased protein content in the conjugate vaccine, when the conjugate antigen is combined to DTPa Hepatitis B, but not when the conjugate is injected alone. This is in clear contrast to the situation observed when the conjugate vaccine is given alone, where the Lower PS-TT ratio (ie more carrier protein) results in a better antibody response to the
15 Polysaccharide (see day 42 anti PS titre of Groups 1 to 3 going from 25, to 18 to 5 µg/ml with a decreasing amount of TT, while they go from .3, 4 to 8 to 19 µg/ml for the same TT contents, but when given in combination with DTPa He B vaccine.

Example 3

20

Effect of TT/PS ratio on compatibility of Hib with DTPa

Groups of 10 rats (OFA, 5 weeks of age, female) were immunised 2 times at 24 days interval by the subcutaneous route with the different TT-PRP vaccines
25 given at 2 dosages: 0.5γ PS/rat (table 2) or 0.05γ PS/rat (table 3). The Hib001 vaccine was reconstituted with saline or 1 dose of DTPa (lot 119) to have a concentration of 10γ PS/ml. The liquid vaccines were diluted with saline or 1 human dose of DTPa to have 10γ PS/ml. The vaccines were then further diluted in saline to have 2.5γ PS/ml or 0.25γ PS/ml. The rats were injected with 200 µl of
30 each vaccine within one hour after dilution.

The animals were bled at day 24 and day 38 and the anti-PRP IgG Abs were measured by ELISA in individual sera and expressed in γ/ml. The GMT was calculated for each group.

Example 4**Comparison of Immunogenicity of DTPa, and DTPa Hepatitis B vaccine mixed with low ratio or 'normal' PRP:TT**

5

Groups of 10 rats (OFA, 1 week of age) were immunised three times (SC) at two week intervals with 0.5 μ g of PS combined 1 hour before injection with 1/20th a human dose of DTPa or DTPa HepB. The animals were bled 2 weeks after the third dose and the antibodies against pertussis toxoid (PT), FHA, pertactin (69k),
10 Diphtheria toxoid (D) Tetanus toxoid (T) and Hepatitis B surface antigen (HBS) measured by Elisa. The titres, expressed as a mid point dilution using a reference are shown in Table 4.

The results of this experiment together with the result from example 3
15 demonstrate that the Hib response is enhanced in a combination vaccine, if the amount of protein in the conjugate is reduced. Moreover, there is no significant interference to the immune response of any other component of the vaccine.

TABLE 1

Conjugate	Ps/Protein Ratio Weight:Weight	Conjugate alone		Conjugate plus Hep B and DTPa	
		D28 (PI)	D42 (PII)	D28 (PI)	D42 (PII)
Buffer		0.05	0.11	0.05	0.11
PRP - TT	1/3	0.5	25	3.4	29
PRP-TT	1/2	0.6	18	8	40
PRP-TT	1/1	0.3	5	19	38

TABLE 2

5

Vaccine	PS/TT	α -PRP (γ /ml)			
		Alone		+ DTPa	
		d24	d38	d24	d38
NaCl	-	0.002	0.01	0.002	0.01
Hib001 A44 (control)	1/2.5	0.08	51.5	0.63	40.3
Dhib015	1/3	0.88	64.1	0.47	19
Dhib026	1/1	0.07	9.1	1.27	66.7

TABLE 3

Vaccine	PS/TT	α -PRP Abs(γ /ml)			
		Alone		+ DTPa	
		d24	d38	d24	d38
NaCl	-	0.002	0.01	0.002	0.01
Hib001 A44 (control)	1/2.5	0.05	7.0	0.09	14.4
Dhib015	1/3	0.07	17.7	0.12	14.7
Dhib026	1/1	0.02	0.45	0.17	51.6

TABLE 4

Vaccine	Ab response after 3 doses (d 42)						
	α -PRP	α -D	α -T	α -PT	α -FHA	α -69k	α -HBS
None (NaCl)	<0.1	<3	<2	<8	<4	<2	<2
DTPa + Hib (Control PS/TT-1/2.5)	3.4	2847	6976	1518	2119- N	127	-
+ Hib (low ratio) (PS/TT ~ 1/1)	20.9	2847	9579	2144	3551- S	35	-
DTPaHB + Hib (PS/TT-1/2.5)	2.8	2001	3622	1603	1123- N	18	3622
+ Hib (low ratio) (PS/TT - 1/1)	11.4	1874	6674	1402	3035- S	12	6674

Claims:

- 1 A combined vaccine comprising a polysaccharide antigen conjugated to a
5 carrier protein, and wherein the carrier protein is also present as free antigen
characterised in that the ratio of polysaccharide to carrier protein in the
conjugate is from 1:0.3 to 1:2.
- 2 A combined vaccine as claimed in claim 1 wherein the carrier protein is a
10 Diphtheria or Tetanus antigen.
- 3 A combined vaccine as claimed in claim 1 or 2 wherein the ratio of
polysaccharide: carrier protein is from 1:0.5 : 1:1.5.
- 15 4 A combined vaccine as claimed in claim 1 wherein the polysaccharide
component is derived from *Haemophilus influenzae* b (Hib) or *Streptococcus*
pneumoniae, or *Neisseria meningitidis* A, B, or C.
- 5 A combined vaccine as claimed in claim 4, further comprising antigens
20 protective against Diphtheria, Tetanus, and pertussis infection.
- 6 A combined vaccine as claimed in claim 4 or claim 5 further comprising an
antigen against hepatitis B virus.
- 25 7 A combined vaccine as claimed in any one of claims 4 to 6 further comprising
an antigen against hepatitis A virus.
- 8 A combined vaccine as claimed in any one of claims 4 to 7 further comprising
an antigen providing immunity against polio
30
- 9 A combined vaccine composition according to any one of claims 4 to 8
comprising an antigen providing immunity to *Streptococcus pneumoniae* and
Haemophilus influenzae B.
- 35 10 A combined vaccine according to any one of claims 4 to 9 additionally
comprising a suitable adjuvant.
- 11 A vaccine as hereinbefore claimed for use in medicine.

- 12 A method of treating a human susceptible to or suffering from an infectious disease, comprising administering an effective amount of a vaccine as claimed in claims 1 to 10

5

- 13 A polysaccharide antigen conjugated to a carrier protein wherein the ratio of polysaccharide to carrier is from 1:0.3 to 1:2.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 96/02436

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K39/385 A61K39/00 A61K39/39

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 594 950 (AMERICAN CYANAMID COMPANY) 4 May 1994	1-5, 11-13
Y	see the whole document	6-10
X	INFECTION AND IMMUNITY, vol. 62, 1994, pages 9-14, XP000602292 BARINGTON, T. ET AL.: "Opposite effects of actively and passively acquired immunity to the carrier on responses of human infants to a Haemophilus influenzae type b conjugate vaccine"	1-5, 11-13
Y	see the whole document	6-10
	--- -/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

18 October 1996

Date of mailing of the international search report

15. 11. 96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+ 31-70) 340-3016

Authorized officer

Olsen, L

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 96/02436

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BIOLOGICALS, vol. 22, 1994, pages 353-360, XP000603003 CORBEL, M.J.: "Control testing of combined vaccines: a consideration of potential problems and approaches" see the whole document	1-5, 11-13
Y	---	6-10
X	BULLETIN DE L'ACADÉMIE NATIONALE DE MÉDECINE, vol. 177, 1993, pages 1381-1387, XP000602905 BÉGUÉ, P.: "Vaccins haemophilus influenzae" see the whole document	1-5, 11-13
Y	---	6-10
X	INFECTION AND IMMUNITY, vol. 61, 1993, pages 432-438, XP000602293 BARINGTON, T. ET AL.: "Non-epitope-specific suppression of the antibody response to Haemophilus influenzae type b conjugate vaccines by preimmunization with vaccine components" see the whole document	13
A	---	1-12
A	US,A,4 644 059 (GORDON LANCE K.) 17 February 1987 see the whole document	1-13
A	---	
A	EP,A,0 208 375 (SCLAVO S.P.A.) 14 January 1987 cited in the application see the whole document	1-13
X,P	---	
X,P	CLINICAL IMMUNOTHERAPEUTICS, vol. 4, November 1995, pages 376-386, XP000602898 FRASCH, C.E.: "Haemophilus influenzae type b conjugate and combination vaccines" see the whole document	1-5, 11-13

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 96/02436

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0594950	04-05-94	AU-B- 669570	13-06-96
		AU-A- 5029093	12-05-94
		CA-A- 2109159	28-04-94
		CZ-A- 9302272	18-05-94
		FI-A- 934725	28-04-94
		HU-A- 67239	28-03-95
		JP-A- 7300427	14-11-95
		NO-A- 933856	28-04-94
		NZ-A- 250029	27-09-94

US-A-4644059	17-02-87	US-A- 4496538	29-01-85
		AU-B- 561978	21-05-87
		AU-A- 1822783	08-02-84
		CA-A- 1210695	02-09-86
		EP-A- 0098581	18-01-84
		JP-A- 5262668	12-10-93
		JP-B- 7121872	25-12-95
		JP-B- 3047253	18-07-91
		JP-T- 59501360	02-08-84
		WO-A- 8400300	02-02-84
		US-A- 4619828	28-10-86

EP-A-0208375	14-01-87	CA-A- 1272952	21-08-90
		DE-A- 3682838	23-01-92
		HK-A- 192895	29-12-95
		JP-B- 7121870	25-12-95
		JP-A- 62030726	09-02-87
		US-A- 4711779	08-12-87
